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Anatomy, palynology and nutlet micromorphology of Turkish endemic *Teucrium sandrasicum* (Lamiaceae)

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Abstract: In this study, the anatomical features of the leaf and stem, besides the pollen and nutlet characteristics of *Teucrium sandrasicum* are investigated. *T. sandrasicum*, belonging to sect. *Teucrium*, is an endemic perennial herb growing on serpentine around Muğla province. The anatomical studies on *T. sandrasicum* revealed that the stem shares the general characteristics of the Labiatae family. The leaves clearly exhibit xeromorphy due to features such as the distribution of stomata on the lower surface (hipostomatic), the occurrence of guard cells below the epidermis (xeromorphic type), inrolled margins, thick cuticle layer, thick outer epidermal cell wall, a high density of trichomes and thick palisade layer of the mesophyll. The anatomical studies showed that the upper epidermal cells of the leaf include many spherocrystals. The pollen grains are prolate, medium in size, 3-colpate with verrucate ornamentation. The nutlets are ellipsoid with a reticulate-verrucate surface. The results have proven that *T. sandrasicum* is different from the other species of the sect. *Teucrium* because of the branched trichomes on the stem and the lack of eglandular trichomes on the nutlets.

Key words: *Teucrium sandrasicum*; Labiatae; anatomy; palynology; micromorphology

Introduction

Teucrium is a large and polymorphic genus distributed mainly in Europe, North Africa and in the temperate parts of Asia. The basic sectional arrangement of the genus is based mainly on the calyx and inflorescence types with varying characteristics (Bentham 1835). The difficulty of classifying some species into sections based on their morphological characters and debates over the affinities of some of their sections led to confusion in determining sectional boundaries and interspecific relationships (Abu-Assab & Cantino 1993). However, the survey on the micromorphology of trichomes of 56 *Teucrium* L. species belonging to the 9 sections of the genus in the Mediterranean area demonstrated the taxonomic value of these micro-characters (Navarro & Oualidi 2000).

The genus *Teucrium* was represented with 27 species in Turkey (Ekim 1982). Duman (2000) mentioned *T. ekimii* H.Duman and *T. ozturkii* A.P.Khokhr. and Dönmez (2006) recorded *T. chasmophyticum* Rech. f. for the Flora of Turkey. The total number of *Teucrium* species in the Turkish flora has reached 30 species by adding these new records.

Earlier works on *Teucrium* pollen were mainly based on LM and TEM (Nabli 1970, 1972). The pollen morphology of 32 Turkish *Teucrium* taxa was investigated by LM (Oybak-Dönmez & İnceoğlu 1988). The pollen morphology in many Labiatae members including *Teucrium* from various parts of the world was investigated under SEM and TEM, and its phylogenetic implications were discussed by Abu-Assab & Cantino (1992). Besides, detailed exine sculpturing of the pollen of some Turkish *Teucrium* L. taxa were determined by SEM. Two main exine sculpturing types, verrucate (in sects. *Teucrium*, *Scordium* Boiss, *Chamaedrys* Benth., *Polium* Benth., *Stachyobotrys* Benth. and *Scorodonia* Benth.) and reticulate (only in sect. *Isotriodon* Boiss.) were defined (Oybak-Dönmez et al. 1999).

T. sandrasicum is an endemic species classified as "Conservation Dependent (LR/cd)" category of IUCN and taxonomically belongs to sect. *Teucrium* (Ekim 1982; Ekim et al. 2000). In this study, we report the anatomical and palynological features and the nutlet's micromorphological characteristics. We also discuss the taxonomic value of these findings with the previous studies on the genus *Teucrium*.

Material and methods

Plant samples of this species were collected from Sandras Dağı in the province of Muğla. The specimens were dried using standard herbarium techniques and stored at the Herbarium of Selçuk University, Faculty of Education and later used for palynological and nutlet surface studies.

Anatomical observations
were performed using an average of 25 specimens fixed in 70% alcohol. Anatomical investigations were carried out on the cross-sections of the upper stems, lower stems, the leaves and surface sections of the leaves. The cross-sections were

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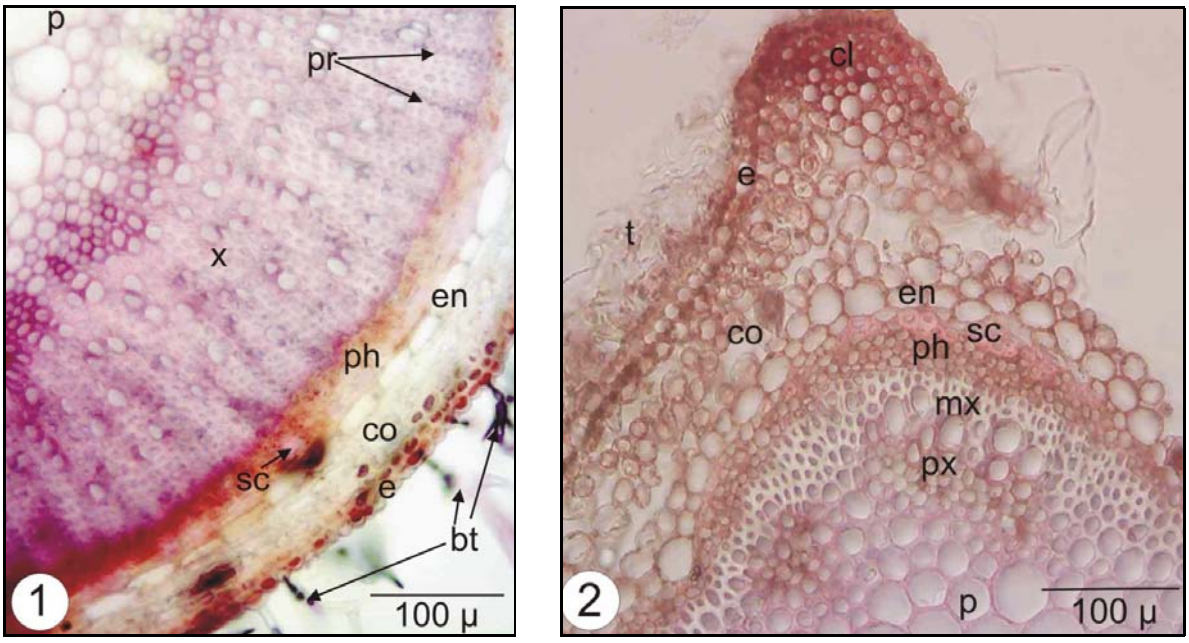

Figs 1–2. Anatomical structure of the stem. 1 – Lower stem, 2 – Upper stem, (bt: branched trichome; e: epidermis; co: cortex; en: endodermis; sc: scleranchyma; ph: phloem; x: xylem; pr: pith ray; p: pith).

dyed with basic fucsin and covered with glycerin-gelatin (Vardar 1987). The photographs of the sections were taken using an Olympus BX-50 microscope. The stomatal index was calculated as described by Meidner & Mansfield (1968).

Palynological investigations
were made using both a light and a scanning electron microscope. For light microscope studies, the pollen slides were prepared according to Wodehouse (1935) technique. Pollen grains were dissected from herbarium specimens and placed on clean microscope slides. Glycerin-gelatin with basic fuchsine was placed on the pollens, melted, and mixed with a clean pin to get scattered pollen grains. Measurements and morphological observations were made using an Olympus BX-50 microscope. The measurements of the polar axis, the equatorial diameter, the colpus length, the exine and the intine thickness for 30 pollen grains were conducted under the light microscope (×400). The palynological data obtained were reported for their mean-size (minimum-maximum). The pollen grains were also placed on stubs directly and covered with gold. After coating, they were observed and photographed with a scanning electron microscope to determine exine ornamentation. Pollen terminology follows Faegri & Iversen (1989) and Punt et al. (1994).

Nutlets
were previously observed using a light microscope to ensure that they were of normal size and maturity. The observations and measurements were made using 25 mature nutlets. The mature nutlets were also mounted directly on aluminum stubs and covered with gold. After coating, they were observed and photographed with a scanning microscope.

Results

Anatomical Properties
Lower Stem: In the lower parts of the stems, the transitional stage from primary structure to secondary structure is visible. The transverse section taken from the lower part of the stem revealed the following: It is circular shaped. The surface is still covered by the epidermis and there are branched hairs on it. Underneath the epidermis, 4–5 layered parenchymatic cortex is located. Parenchymatic cells are rectangular and flattened. Endodermis is distinguishable and composed of a single layer of rectangular cells. There are scleranchyma fibers forming bouquets between the endodermis and the phloem elements. Cambium is indistinguishable. The xylem is composed of trachea and tracheids. The pith rays are 1–2 layered. The pith is present at the middle of the stem, and completely filled up with large orbicular parenchymatic cells (Fig. 1).
Upper Stem: The transverse section taken from the upper part of the stem revealed the following: It is rectangle shaped. The epidermis consists of rectangular and ovoidal cells forming a single layer and is surrounded by a cuticle layer. There are long and thin-walled multicellular hairs on the epidermis. Underneath the epidermis, there is collenchyma with a single layer of cells between the corners but 6–7 layers of collenchyma in the corner of the stem. The shape of the collenchyma cells is ovoid. The cortex, consisting of 5–6 layered ovoidal parenchymatous cells, is located under the collenchyma. The endodermis is distinct and composed of a single layer of rectangular cells. The pericycle is present below the endodermis and it is made up of 1–2 layered scleranchymatous cells encircling the vascular bundles from the outside. The vascular bundles at the corners are larger than the others. Cambium is indistinguishable. The phloem and the xylem members are clear. Protoxylem and metaxylem members are distinguishable. The tracheal elements of the protoxylem are larger than those of the metaxylem. The pith is present at the middle of the stem, and is completely filled up with large orbicular parenchymatic cells (Fig. 2).

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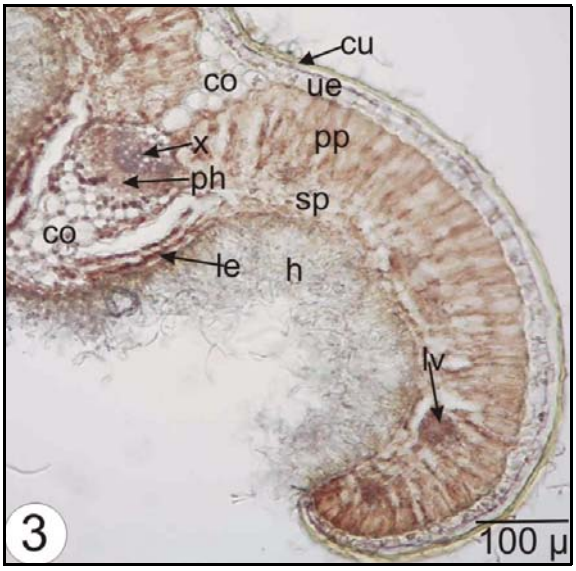


Fig. 3. Transection of the leaf (cu: cuticle; ue: upper epidermis; co: collenchyma; pp: palisade parenchyma; lv: lateral vascular bundle; sp: spongy parenchyma; x: xylem; ph: phloem; le: lower epidermis; h: hair – trichome).

Leaf: The transverse section of the lamina and surface preparations of both epidermises revealed that the upper and lower epidermises comprise uniseriate, oval and rectangular cells. The upper epidermis cells are larger than the lower ones. Both epidermises are covered with a cuticle. The upper cuticle layer is fairly thicker than the lower one. There are long and thin-walled multicellular hairs on both epidermises, but the trichome density of the upper epidermis is not as high as that of the lower epidermis. The walls of the lower epidermis cells are corrugated but those of the upper one are not. Midrib is triangle shaped and has 4–6 layered collenchyma located below both epidermises. Vascular bundles are collateral and surrounded by a parenchymatic bundle sheath. Leaves are bifacial (dorsiventral) and hipostomatic. The stomata are anomocytic and the guard cells form a row below the epidermis (xeromorphic type). Palisade parenchyma cells are 2-layered under the upper epidermis. Spongy parenchyma cells are 2–3 layered under the lower epidermis. However, the palisade parenchyma occupies about 65% of the mesophyll. Namely, the palisade tissue is about two times as thick as the spongy tissue (Figs 3, 4, 5).

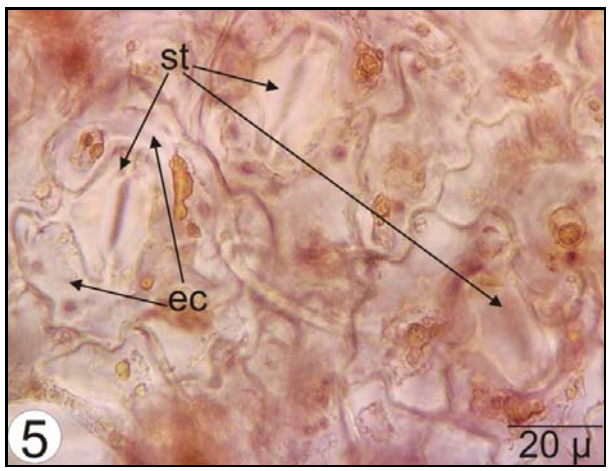
The number of stomata is 78 ± 4 and that of epidermis cells 384 ± 6 in mm^2 on the lower epidermis of the leaf. The stomata index is 16.88 for the lower epidermis. The stomata are 35–40 × 25–30 μm. The upper surface of the leaf lacks stoma. The upper epidermis cells have thick walls and include spherocrystals. The pit pairs can be seen on the upper epidermis cells walls.

Pollen characteristics: The pollens of *T. sandrasicum* are radially symmetrical, isopolar and 3-zonocolpate. The size of pollen grains (polar axis × equatorial axis) is 41.5 μm (38–45 μm) × 20 μm (18–22 μm). The ratio of P/E is 1.7–2.0 (prolate in shape). The outline is elliptic in equatorial view and circular in meridional optical section. The exine thickness is 1.70 μm (1.60–1.90 μm) and intine thickness 0.70 μm (0.60–0.80 μm). Exine sculpturing is verrucate. The number of wart per μm^2 ranges from 3–6 (Figs 6, 7).

Nutlet morphology: The nutlets are ellipsoid, 4.0–4.5 mm long, 2–2.5 mm wide and with a reticulate-verrucate surface. The surface densely bears sessile oil glands forming verrucation, has small projection forming micro-verrucation and lacks eglandular trichomes (Figs 8, 9).

Discussion

Metcalfe & Chalk (1950) pointed out that the stems of the family Labiatae species are rectangular and the collenchymatic tissue covers a broad area at the corners and scleranchymatic tissue surrounds the vascular tissue. The anatomical studies on some of the family Labiatae species showed that they have the same anatomical characteristics (Kaya et al. 2000; Kandemir 2003; Uysal 2003). These characteristics are observed



Figs 4–5. Surface sections of the leaf. 4 – Upper surface, 2 – Lower surface, (ec: epidermal cell; sc: spherocrystal; p: pit; st: stoma).

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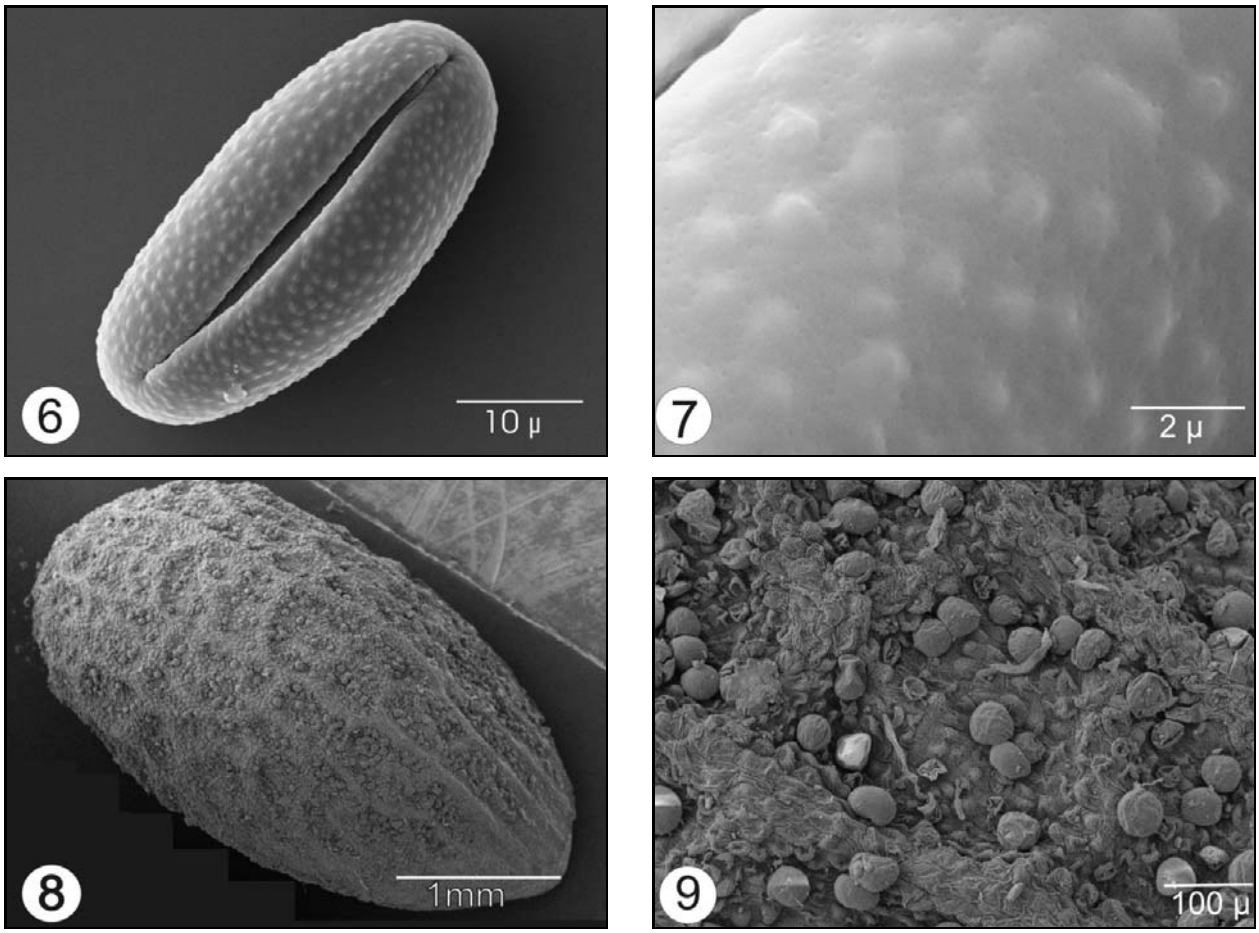

Figs 6–9. SEM photos of the pollen and nutlet. 6 – Equatorial view of the pollen with an aperture, 7 – Pollen surface in detail showing verrucate exine sculpturing, 8 – General appearance of the nutlet, 9 – Nutlet surface in detail.

in *T. sandrasicum* as well. In addition, the spherocrystals occur in the upper epidermis cells of the leaf in this species. This crystal shape is recorded for the first time in the genus *Teucrium*. Whereas, solitary crystals and druses are the most common crystal types amongst dicotyledons, other crystal types such as spherocrystal are rarely observable. The more restricted occurrence of the spherocrystal enhances its diagnostic value (Metcalfe 1983). Further studies may clarify the taxonomic value of the crystal shape in the genus *Teucrium*.

The characters, traditionally accepted as evidence that the plants possessing them are xerophytes, were recorded previously (Metcalfe & Chalk 1983; Yakar & Bilge 1987; Öztürk & Seçmen 1996). The leaves of *T. sandrasicum* show many structural features that could be interpreted as typically xeromorphic, such as the epidermal cells in which the outer walls and overlying cuticle are thick, a high proportion of typical palisade tissue, a small body with inrolled margins. Moreover, they are hipostomatic with stomata concealed by dense and white trichomes and occurring below the epidermis (xeromorphic type). Therefore, *T. sandrasicum* is clearly a xerophytic species.

Some Turkish *Teucrium* pollens were determined by SEM (Oybak-Dönmez et al. 1999). They showed two main exine sculpturing types. The taxa from the sect. *Isotriodon* show reticulate pollen exine sculpturing, those from the sects. *Teucrium*, *Scordium*, *Chamaedrys*, *Polium*, *Stachyobotrys* and *Scorodonia* exhibit verrucate pollen exine sculpturing. The verrucate pollen exine sculpturing in the sect. *Teucrium* species is confirmed by the results obtained from this study.

The presence of branched hairs in vegetative structures has a high systematic value. Branched nonglandular hairs are confined to sect. *Polium* subsect. *Polium* and also present in one rare endemic species of sect. *Chamaedrys* from Morocco, *T. barbarum* and another isolated endemic species from Canary Islands, *T. heterophyllum* L'Hér (Navarro & El Oualidi 2000). These hairs are also found on the lower part of the stem in Turkish local endemic *T. sandrasicum* from sect. *Teucrium*. The branched hairs are recorded for the first time in the sect. *Teucrium*.

The presence or absence of trichomes and oil glands on the nutlet surface is also among the most useful taxonomic characters in *Teucrium* L., and can be used as a taxonomic marker in the infrageneric classification of the genus. The systematic relationship between sects. *Chamaedrys* and *Teucrium* were established by Marín et al. (1994) on the basis of the presence of the hairy nutlets. However, sect. *Teucrium* is well defined not only by the densely distributed eglandular trichomes but also by the presence of oil glands on nutlets (Marín et al. 1994). *T. orientale* subsp. *taylori* from sect. *Teu-*

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crium shows hairy nutlets similarly to members of sect. *Chamaedrys* and has sessile oil glands and reticulate ridges on its nutlets (Navarro & El Oualidi 2000). *T. sandrasicum* from sect. *Teucrium* has densely sessile oil glands as well, but lacks eglandular hairs on the nutlets. This, together with the presence of branched hairs on its stem, is the most distinguishing feature of *T. sandrasicum* from other species of sect. *Teucrium.*

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